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ORIGINAL ARTICLE

Variation of indole acetic acid (IAA) amounts in cambial-region tissues in 7- and 24-year-old sugi (*Cryptomeria japonica*) trees

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Abstract Recently, breeding programs have attempted to produce high growth rates for shorter rotation cycles in plantation trees. In these trees, the ratio of juvenile wood increases; thus, the juvenile wood properties should be improved for structural use. To this end, it is important to understand the influences on juvenile wood properties precisely. In this study, we report on the indole acetic acid (IAA) amounts of juvenile sugi (*Cryptomeria japonica*) trees in September and compare the IAA amounts to those in mature trees. The IAA amounts at the lower trunks in juvenile trees were significantly larger than those in mature trees and the IAA amounts decreased with tree height. In each stand, except a mature tree stand, there is no significant effect of IAA amounts on latewood width and MFA. However, put together all samples, the latewood width and MFA increased with IAA amounts in samples with IAA <200 ng/cm^2. The samples at lower trunk in juvenile trees had significantly larger IAA amounts, larger MFA and larger latewood width than the samples in mature trees ($p < 0.01$). The very large IAA amounts may have a certain relation with juvenile wood properties.

Keywords Sugi · IAA · Crown-formed wood · MFA

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Introduction

Softwood is one of the most important renewable resources in the world and is mainly used for the structural components of wooden structures. In generally, the properties of softwood vary dramatically in the radial direction (i.e., from pith to bark). Juvenile wood is the wood formed nearest the pith, usually in an area where there are rapidly changing wood properties such as specific gravity, cell length, cell wall characteristics, chemistry, and cell orientation [1, 2]. Because of the negative effects on the performance of the final products, many studies in economically important conifers have been done to understand juvenile wood properties and to elucidate the effects of these properties on the final products [3, 4]. Recently, tree-breeding programs have attempted to increase growth rates of plantation trees for shorter rotation cycles. The logs from plantations with higher growth rates and shorter rotation cycles are assumed to have a larger juvenile wood ratio than logs from conventional plantations. Therefore, studies on the improvement of juvenile wood properties of radiata pine (*Pinus radiata*) [5, 6] and slash pine (*Pinus elliottii*) [7] have been done to ensure superior structural materials through a shorter rotation age. To improve the properties of juvenile wood efficiently, we have to understand the various influences on juvenile wood growth more precisely.

Some researchers believe that the formation of juvenile wood is related to the year of formation (ring number) of the cambial initials and the formation of a somewhat cylindrical, or a conical zone in the center of the tree while other researchers think that distance from the pith is a better measure for defining juvenile wood than ring number [8]. In yet another concept, some researchers use the term "crown-formed wood" in relation to the position of the wood from the crown during the formation of the wood [9]. In Corsican pine (*Pinus nigra*), it was suggested that the term "crown-formed wood" should be used to describe fluctuations in wood structure (density) associated with the size of the crown, superimposed upon the inherent trends due to cambial age [10]. The crown size and the position of the wood from the crown when the wood was formed may affect the radial variation of wood properties.

Studies on xylem formation have focused on the role of plant growth regulators, especially indole acetic acid (IAA), in wood formation [9, 11–15]. It has been assumed that IAA is actively synthesized in elongating shoot apices and is transported to the stem cambium, where it then stimulates tracheid production. Based on these studies, IAA is believed to be an important regulator in xylem formation in conifers. In a previous study on mature sugi trees (*Cryptomeria japonica*, Japanese cedar), we reported that the distance from the crown and the crown length ratio (CL ratio) affect the IAA amounts in the cambial-region tissues in the stem [16]. As the trees in sugi plantations grow from juvenile to mature trees, the lower branches naturally wither and fall due to competition between trees, and the crown then moves from a lower position to a higher position in the trunk, and CL ratio, i.e., the ratio between the crown length to the total tree height, decreases. Based on the results obtained in the previous study, it can be assumed that in juvenile trees, in which the lower branches near the ground are alive and the CL ratios are close to 100 %, the absolute values and longitudinal variation patterns of the IAA amounts might be quite different from those in mature trees. These ideas support the concept of "crown-formed wood" described above. To elucidate the processes involved in juvenile wood formation, the IAA amounts in the cambial-region tissues in juvenile trees should be examined and compared to those in mature trees. In addition, the effect of IAA amounts on xylem formation in juvenile trees and the variation of wood properties in juvenile and mature trees should be evaluated. In radial variation of wood properties of sugi, variation of microfibril angle (MFA) is very important, because wood density in juvenile wood is larger than mature wood. However, there have not been enough quantitative studies on IAA in cambial-region tissues in juvenile sugi trees.

The objectives of the present study were to examine: (1) the absolute value of the IAA amounts in the cambial-region tissues at the lower and upper trunks of juvenile and mature trees; (2) the effects of IAA amounts on cambial growth in juvenile trees; (3) the effects of IAA amounts in the cambial-region tissues on the variation of latewood width and latewood MFA of the middle layer in the secondary wall of the tracheids in juvenile and mature sugi trees.

Based on the seasonal variation of IAA amounts found in pine trees [12, 13], the IAA amounts in sugi trees were assumed to vary seasonally, and data regarding the seasonal variation of IAA amounts may be important. However, in the present study, we focused on the IAA amounts present in September, because these IAA amounts represented the amounts present during latewood formation. Latewood contributes much more than earlywood to the mechanical properties of softwood. Therefore, we compared the September IAA amounts in juvenile trees to those in mature trees.

Materials and methods

Sample trees and samples for IAA quantification

In the present study, we estimated the wood of ring number $\leq$10 and the wood of ring number $\geq$11 as juvenile wood and mature wood, respectively. As shown in Table 1, fifteen juvenile trees (7 years old, stand A) and thirteen mature trees (24 years old, stands B and C) were selected as sample trees. Therefore, in juvenile trees (stand A trees), juvenile wood is formed in the entire tree. In mature trees (stand B and C trees), it was assumed that mature wood is formed in the trunk below the crown base. These stands consisted of some obi-sugi cultivars (a popular sugi cultivar group in southern Kyushu, Japan), but there was no information on which cultivars were planted at these stands. These trees had been planted in sugi stands for wood production in the experimental forest of Miyazaki University. No silvicultural practices (thinning, pruning) were carried out at these stands. The average annual temperature and precipitation at the experimental forest at Miyazaki University from 2001 to 2011 were 17.3 °C and 2793 mm, respectively. The altitude of the stands used in the present study ranged from 100 to 350 m. The diameter at breast height (DBH) and the tree height were measured with a tape measure and ultrasonic hypsometer (Vertex III, Haglof, Inc), respectively.

The crown base in juvenile trees (stand A) was near the ground, although the crown in mature trees (stands B and C) was far from the ground.

For the measurement of the IAA amounts in cambial-region tissues, samples (3 cm (T) × 5 cm (L) × 1 cm (R)) of cambial-region tissues sandwiched by the outer bark and the outermost wood were obtained from the sample trees listed in Table 1. To meet objective (1), the samples of juvenile trees were obtained from a point 2.5 m above the ground (upper trunk) and from a point 1.2 m above the ground (lower trunk) in stand A sugi trees in September 2007 (2 positions × 15 trees, for a total of 30 samples). The samples of mature trees were obtained from a location at a distance above the ground of 3/5 of the crown height (upper trunk) and from a point 1.2 m above the ground

Table 1 Sample trees

Stand	age	*n*	Density (trees/ha)	DBH (cm)	Tree height (m)	CL ratio (%)	Crown width at BH (m)	LFR at sampling date (%)
A	7	15	3000	9.6 (3.1)	5.8 (0.9)	78.7 (3.5)[a]	2.03 (0.37)	54.8 (19.6)
B	24	6	3600	15.3 (1.8)	12.2 (1.1)	43.7 (1.2)	–	55.3 (14.1)
C	24	7	2400	22.7 (3.1)	17.1 (0.8)	39.3 (5.1)	–	55.3 (20.1)

The value of DBH, tree height, CL ratio, crown width at BH and LFR at sampling date represent the average of sample trees and the values in parentheses represent the standard deviations. *Crown width at BH* crown width at breast height *LFR at sampling date* the ratio of the width of latewood formed before sampling date (September) to the total latewood width (%). LFR at sampling date of the lower and upper stem of each stand trees was averaged. Seven samples in A stand were excluded from calculation of LFR at sampling date because these samples were not in latewood formation

n number of sample trees *DBH* diameter at breast height, *CL ratio* the ratio between the crown length to the total tree height [a]we decided the height of crown base of juvenile trees as 1.2 m, because the branches below breast height do not affect the IAA amounts of samples

(lower trunk) in stands B and C trees in September 2004 (2 positions × 5 or 6 trees × 2 stands, for a total of 22 samples). In addition, to examine the amounts of IAA in the cambial-region tissues of the crown in mature trees, two trees were felled and samples were obtained at 5 locations with different heights above the ground from one tree at each stand (stands B and C) in September 2006 (5 positions × 1 tree × 2 stands, for a total of 10 samples). Samples for IAA quantification were stored in liquid nitrogen before extraction.

Measurements of IAA amounts in cambial-region tissues

IAA in cambial-region tissues was identified and quantified by liquid chromatography/mass spectrometry (LC/MS/MS or LC/MS). Samples were homogenized and extracted in 1 h by methanol with antioxidant medium (0.02 M diethyldithiocarbamic acid, Wako, Ltd.). Methanol extraction was repeated three times at 4 °C in darkness. For the quantification of IAA amounts, 1 μg of deuterium IAA (Sigma Co., Ltd, D_2-IAA, 97 % contents) was added to the methanol for extraction as an internal standard. The extracts were evaporated and the residues were dissolved in 10 ml distilled water, and then the aqueous solutions were adjusted to pH 2.5 by formic acid. The supernatants were obtained from the aqueous solutions by centrifugal separation, loaded onto reverse-phase cartridges (sep-pack cartridge, C18 500 mg, Waters), and eluted with 1 ml 80 % methanol adjusted to pH 2.5. The effluents were subjected to LC/MS/MS or LC/MS.

LC/MS/MS analysis was carried out using a liquid chromatograph (2695, Waters) coupled to a quadrupole mass spectrometer (Q-micro, Waters) with an ion source operated in ESI mode. The column was a TSK gel ODS-100 V (100 mm × 2.0 mm, TOSOH), and the flow rate was 0.2 ml/min. As the mobile phase, 45 % methanol containing 0.5 % acetic acid was used. The mass spectrometer was run in positive-ion mode and multiple-reaction-monitoring (MRM) mode. For the identification of endogenous IAA, the retention time on the liquid chromatograph, the parent ion (*m/z* 176 (M^+)) and fragment ions (*m/z* 76.9, *m/z* 103, *m/z* 130) of authentic IAA (Merck, 99 % contents) were used. For the quantification of endogenous IAA, the sensitivity of LC/MS/MS was tuned using authentic IAA, and a calibration curve was obtained from an analysis of samples of D_2-IAA and authentic IAA at different mixing ratios. From the MRM chromatogram of endogenous IAA (parent ion: *m/z* 176, fragment ion: *m/z* 130) and D_2-IAA (parent ion: *m/z* 178, fragment ion: *m/z* 132), the peak area ratio (area of endogenous IAA/area of D_2-IAA) was obtained, and then the IAA amounts of the samples were calculated using the calibration curve from the obtained peak area ratio [16].

LC/MS analysis was carried out using a liquid chromatograph (M-8093, Hitachi) and atmospheric pressure ionization (API) (M-2008, M-2508, M-2029, Hitachi) coupled to a double-focusing mass spectrometer (M-2000AM, Hitachi). The column was a STR ODS-II (250 mm × 4.6 mm, Shinwa), and the flow rate was 0.7 ml/min. As the mobile phase, 45 % methanol containing 0.5 % acetic acid was used. The mass spectrometer was run in positive-ion mode and single-ion-monitoring (SIM) mode. For the identification of endogenous IAA, the retention time on LC, the parent ion (*m/z* 176) and fragment ions (*m/z* 76.9, *m/z* 103, *m/z* 130) of authentic IAA (Merck, 99 % contents) were used. For the quantification of endogenous IAA, the sensitivity of LC/MS was tuned using authentic IAA, and a calibration curve was obtained from an analysis of samples of D_2-IAA and authentic IAA at different mixing ratios. From the SIM chromatogram of endogenous IAA (*m/z* 176) and D_2-IAA (*m/z* 178), the peak area ratio (area of endogenous IAA/area of D_2-IAA) was obtained, and then the IAA amounts of the samples were calculated using the calibration curve from the obtained peak area ratio.

The IAA amount (ng) per sample weight (g) was affected by the increase in sample weight during xylem

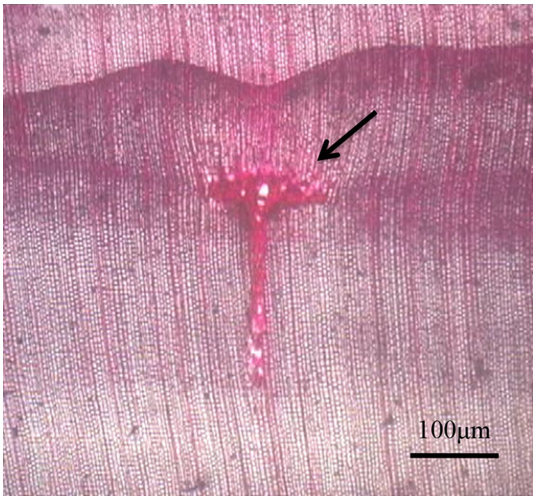

Fig. 1 The position of sampling date in latewood of a juvenile tree. The wound tissues formed by pin insertion to cambial-region tissues were observed (*arrow*)

formation [17]. Therefore, the IAA amount of the cambial-region tissues was shown as the IAA amount (ng) per cambium area ($L \times T$ cm^2) (ng/cm^2). LC/MS/MS analysis and LC/MS analysis were used for juvenile and mature tree samples, respectively.

Observation of cambial growth in juvenile and mature trees at sampling date

To meet objective (2), cambial growth in juvenile trees (stand A trees, Table 1) at a point 2.5 m above the ground (upper trunk) and a point 1.2 m above the ground (lower trunk) were examined in September 2007 by the pinning method [18]. The cross sections of the wound tissues formed by pin insertion to the cambial-region tissues were obtained (Fig. 1). The sites of cambial initials at the time of pinning were determined by observation of the increasing tracheid row at the wound tissues. Based on the sites of cambial initials at the time of pinning, the width of xylem formed after sampling (μm) was measured. In mature trees, xylem formation at sampling date was examined from the observation of the cross sections of samples for IAA measurements.

Measurements of wood properties

To meet objective (3), the latewood width and the MFA of the latewood in the outermost rings were examined in the trees listed in Table 1. Samples were obtained at the upper and lower trunks of each tree to examine the relationship between IAA amounts in September and the anatomical latewood properties. The MFA was measured by the iodine-staining method [19]. I_2 crystallized in gaps between microfibrils in tangential sections of each ring, and the sections were observed with a light microscope. Under light microscopy, MFA was measured using image analysis software (Image J [20]). The MFA of each ring was obtained by averaging the measurements of 30 tracheids. On the cross sections of each ring, the latewood width was measured at 10 radial files in each ring, and the average values were calculated.

Statistical analysis

The differences of the IAA amounts of trees in the three stands and between the upper and lower regions of the trees were examined by one-way ANOVA and multiple comparisons tests (Tukey's HSD test and Bonferroni test) (statistical analysis software, SPSS ver. 16 with Regression and Advanced Models).

Results

IAA amounts in juvenile and mature trees

The IAA amounts in cambial-region tissues in September at the lower and upper trunks of juvenile and mature sugi trees are shown in Fig. 2. In juvenile trees (stand A), the average IAA amounts at the lower and upper trunks were 1157 ng/cm^2 and 386 ng/cm^2, respectively. In mature trees, the average IAA amounts at the lower and upper trunks in stand B trees were 34 and 69 ng/cm^2, respectively, and those in stand C trees were 29 and 26 ng/cm^2, respectively. Juvenile trees had much larger average IAA amounts at both the lower and upper trunks than mature trees. One-way ANOVA showed a significant difference between the IAA amounts at the lower and upper trunks of all three stands of trees ($p < 0.01$). As shown in Fig. 2 and Table 2, the IAA amounts at the lower trunk in stand A trees were significantly larger than those at both the lower and upper trunks in stand B and C trees (multiple comparisons, $p < 0.01$), although those at the upper trunk in stand A trees were not significantly different from those at both the lower and upper trunks in B and C stand trees. As shown in Fig. 2, there was a significant negative correlation between tree height and IAA amounts in all samples at the lower trunks ($r = -0.58$, $p < 0.01$). In mature trees (B and C stand), there was no significant negative correlation between tree height and IAA amounts in all samples at the lower trunks, although there was a significant negative correlation in all samples at the upper trunks ($r = -0.76$, $p = 0.01$).

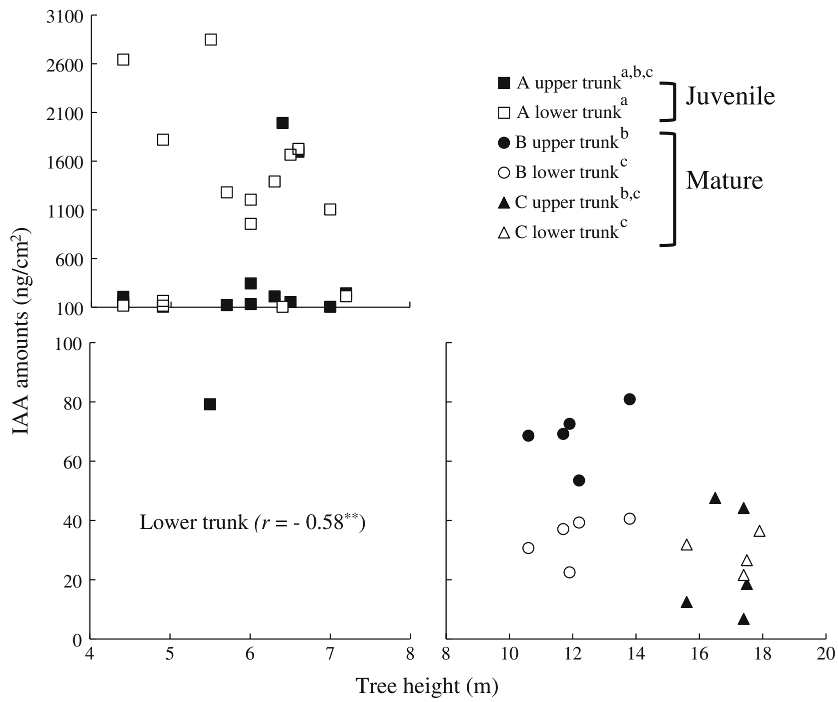

Fig. 2 Tree heights and the IAA amounts in September. There was a negative correlation between tree height and the IAA amounts in all samples at lower trunks. The *different characters a*, *b*, *c* means significant difference among IAA amounts at upper and lower trunk of trees in three stands. $^{**}p < 0.01$

Table 2 MFA and latewood width of sample trees

Stand	Position	IAA amounts (ng/cm^2)	MFA (degree)	Latewood width (mm)
A	Lower	1157 (900)^{a}	25.0 (3.4)^{a}	0.80 (0.32)^{a}
	Upper	386 (598)^{a,b,c}	23.9 (5.0)^{a}	0.73 (0.38)^{a}
B	Upper	69 (10)^{b}	19.4 (1.8)^{a,b}	0.31 (0.12)^{a,b}
	Lower	34 (7)^{c}	19.6 (1.9)^{a,b}	0.39 (0.15)^{a,b,c}
C	Lower	29 (6)^{c}	17.7 (2.3)^{b}	0.27 (0.12)^{c}
	Upper	26 (19)^{b,c}	16.6 (1.2)^{b}	0.28 (0.11)^{c}

The value of IAA amounts, MFA and latewood width represent the average of each position of sample trees and the values in parentheses represent the standard deviations. Average values with different letters are significantly different ($p < 0.05$)

MFA microfibril angle of S_2 layer of latewood tracheids

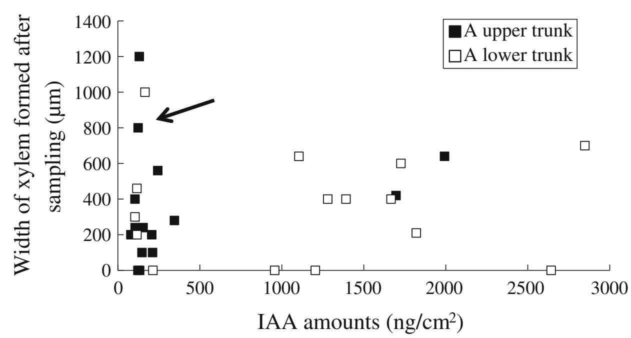

Fig. 3 Effect of IAA amounts on the cambium activity of trees in A stand. Xylem formation in six samples with larger IAA amounts ceased at sampling date (the value of longitudinal axis showed 0), although earlywood formation was observed in a sample with smaller IAA amounts (*arrow*)

Effects of IAA amounts on the xylem formation in juvenile trees

To elucidate the role of IAA amounts in xylem formation in juvenile trees, the relationship between IAA amounts and the width of xylem formed after the sampling date (September) is shown in Fig. 3. In all samples except seven, latewood was being formed in September. The ratio of the latewood width formed before sampling date (September) to total latewood width is shown in Table 1. The average value of the ratio of 14 juvenile trees was 54.8 %. Therefore, it was assumed that xylem formation at the sampling date (September) was in the mid season in latewood formation. In one of the seven samples, earlywood was being formed, while xylem formation had ceased in the other six samples. The results in Fig. 3 suggest there is no clear role of IAA amounts in cessation of xylem formation, frequency of tangential division of fusiform initials, or the earlywood-to-latewood transition in juvenile trees.

Effects of IAA amounts on the variation of latewood width and MFA of latewood in juvenile and mature trees

In the present study, IAA amounts in the lower trunk were observed to diverge sharply between juvenile and mature

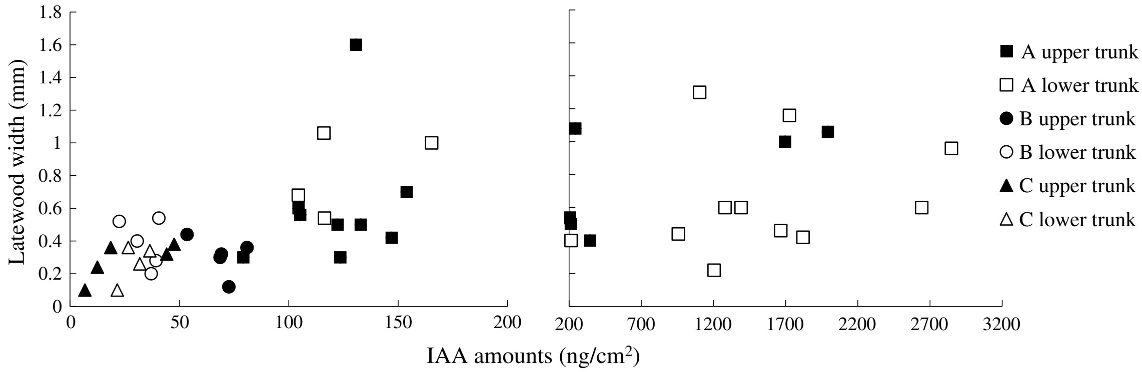

Fig. 4 Effect of IAA amounts on latewood width

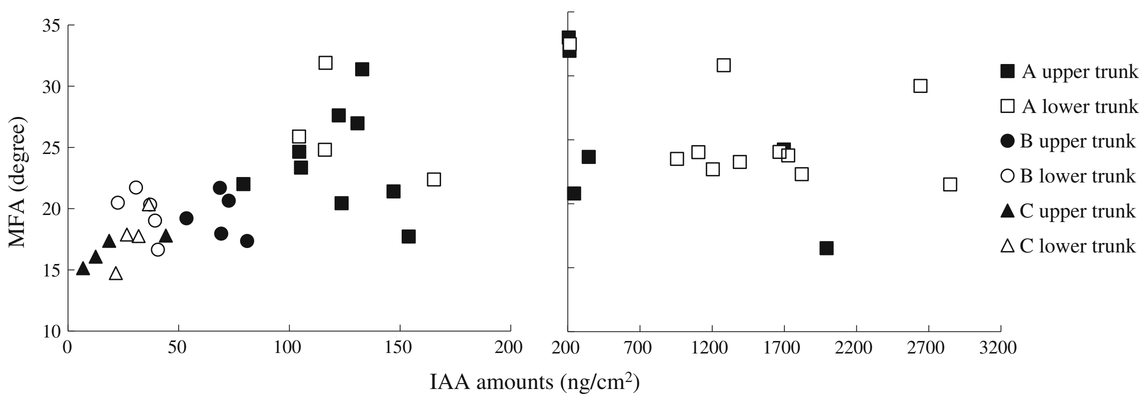

Fig. 5 Effect of IAA amounts on MFA of latewood. *MFA* microfibril angle of S_2 layer in secondary wall of tracheid

sugi trees. Therefore, we tried to elucidate the effects of IAA amounts on the difference of wood characteristics in juvenile and mature trees. As shown in Table 1, the ratio of the latewood width formed before sampling date (September) to total latewood width was calculated. The average value of the ratio of A, B and C stand was 54.8, 55.3 and 55.3 %, respectively. Therefore, it was assumed that xylem formation at sampling date (September) was in the mid season in latewood formation. In Table 2, average values of MFA and latewood width of samples of each stem position of three stands were shown. The order of each stem positions of three stands in Table 2 is decided according to the average values of IAA amounts. Based on the average values, samples with larger IAA amounts assumed to have larger MFA and wider latewood. In A stand, although the upper trunk had smaller cambial age than lower trunk, the IAA amounts, latewood width and MFA in upper trunk were smaller than those in lower trunk. As shown in Table 2, the samples at lower trunk in A stand had significantly larger IAA amounts, larger MFA and larger latewood width than the samples at lower and upper trunk in C stand (multiple comparisons, $p < 0.01$). The relationships between the IAA amounts and the wood characteristics (latewood width and MFA) of latewood in juvenile and mature trees are shown in Figs. 4 and 5. It was assumed that the tendency of relationships between IAA amounts and wood characteristics changed around the IAA amounts of 200 ng/cm^2. The latewood width and MFA assumed to increase with IAA amounts in samples with IAA <200 ng/cm^2. In Fig. 4, there was a significant positive correlation between IAA amounts and latewood width in all samples ($r = 0.45$, $p < 0.01$). However, in each stand, there was no significant positive correlation between IAA amounts and latewood width. In Fig. 5, there was no significant positive correlation between IAA amounts and MFA in all samples. However, in C stand, there was a significant positive correlation between IAA amounts and MFA ($r = 0.71$, $p < 0.05$).

Discussion

From the results obtained in the present study, it was demonstrated that the IAA amounts at lower trunk in juvenile sugi trees in September were very large (Fig. 2). It has been previously reported that the IAA amounts of cambial-region tissues in the trunks of *Pinus densiflora* (20 years old, September), *Pinus sylvestris* (20 years old, May to August) and *Pinus sylvestris* (43 years old, June) ranged from 50 to 200 ng/cm^2 [21], from 40 to 120 ng/cm^2 [17], and from 33 to 168 ng/cm^2 [15], respectively. Therefore, in comparison with mature pine trees, it was recognized that the IAA amounts at the lower trunk in juvenile sugi trees (from 104 to 2848 ng/cm^2) in September were very large. In the introduction, we hypothesized that the absolute values and longitudinal variation patterns of the IAA amounts in juvenile trees might be quite different from those in mature trees. These results obtained in the present study were consistent with our hypothesis. However, only 15 juvenile trees in a stand were examined in the present study. More juvenile sugi trees in other stands should be examined.

In the introduction, we described the concept of "crown-formed wood" [9]. However, in the concept of "crown-formed wood", the difference between the xylem formations at the crown in juvenile and mature trees was not discussed [9]. In Scots pine (*P. sylvestris*, 43 years old, June), IAA amounts at the 9th internode and crown base (IAA amounts at crown in mature trees) ranged from 50 to 150 ng/cm^2 and 50 to 125 ng/cm^2, respectively [15]. As shown in Fig. 2, the IAA amounts at the crown in juvenile trees were assumed to be larger than those at the crown in mature pine trees. To compare the IAA amounts at the crown in juvenile sugi trees to those of mature sugi trees, we preliminary examined the longitudinal variation of IAA amounts in cambial-region tissues in the crown of one tree from each stand B and C in September. The IAA amounts of cambial-region tissues at the crown ranged from 16 to 60 ng/cm^2. Although more number of mature sugi trees should be examined, the IAA amounts at the crown in mature sugi trees may be less than those in juvenile sugi trees. If this assumption was true, because of the small IAA amounts at the crown in mature sugi trees, it was assumed that the reason of very large IAA amounts at the lower trunk in juvenile trees was not the short distance from the crown. IAA amounts in cambial-region tissues are the result of transportation and metabolism of IAA in the cambium. In juvenile trees, the crown base is near the ground; therefore, IAA synthesized at the crown is directly transported to the root system. The exogenous IAA applied at apical bud moved into the root system and reached the apical part of root [22]. The application of exogenous IAA inhibited the root growth, because the optimal IAA concentration for root growth was lower than those in stem tissues [23]. The transportation and metabolism of IAA in root system may be different from that in stem tissues. In comparison with taller mature trees, IAA transportation in shorter juvenile trees may be inhibited by the root system, and inhibited more severely at the lower trunk, possibly causing IAA amounts at the lower trunk to increase, as in a traffic jam. The cause of the very large IAA amounts observed in the present study at the lower trunk in juvenile trees should be studied in the future for better understanding of juvenile wood formation.

The result of the present study suggested that IAA amounts in the lower trunk decreased with tree height (Fig. 2). However, in mature trees (stand B and C), there was no relationship and a significant relationship between tree height and the IAA amounts in lower and upper trunk, respectively. As the trees in sugi plantations grow from juvenile to mature trees, the lower branches naturally wither and fall due to competition between trees, and the crown then moves from a lower position to a higher position in the trunk. As described in introduction, it was assumed that IAA was actively synthesized in elongating shoot apices and transported to the stem cambium. Therefore, it was assumed that tree height was related to crown size. In mature trees, the distance from the crown also may affect the IAA amounts in the cambial-region tissues. It was assumed that after the tree is high enough, and the distance to crown is long enough, the height of the tree does not anymore affect the IAA concentration in the lower trunk. In the upper trunk, although a significant effect of tree height was recognized, growth conditions of these stands may have an effect on IAA amounts.

As shown in Fig. 3, there is no clear role of IAA amounts on xylem formation in juvenile trees. As shown in Figs. 4 and 5, in juvenile trees (stand A trees), there were no significant effects of IAA amounts on latewood width and MFA. In all mature trees except stand C trees in Fig. 5, there were no significant effects of IAA amounts on latewood width and MFA. These results are consistent with those of previous studies on endogenous IAA of mature pine trees [24]. From these results, it was assumed that IAA amounts had no direct effect on xylem formation in each stand. However, in comparison with juvenile and mature trees (Table 2) instead of the relationships in each stand, it was recognized that samples with very large amounts of IAA had wider latewood width and larger MFA than the samples with small amounts of IAA. It was difficult to eliminate the assumption that the very large IAA amounts may have some effects on latewood width and MFA in juvenile wood. In the studies with application of exogenous IAA, the effects of IAA amounts on xylem formation were examined in comparison with branches debudded (very small IAA amounts) and branches debudded and treated apically with exogenous IAA (very large IAA amounts) [11, 14]. In these studies with wide range of applied IAA amounts, an increase in the internal IAA level was positively related to increased tracheid production. In tracheid differentiation, it was also reported that applied exogenous IAA during latewood formation induced earlywood formation, and high concentrations of IAA induced compression wood (large MFA) formation in conifers [9]. In our study, the difference of IAA amounts between juvenile and mature trees was very large, although the difference of IAA amounts of trees in each stand was small (Table 2). Therefore, we assumed that although IAA is essential for xylem formation, the effects of IAA amounts on xylem formation might be significantly recognized only when the IAA amounts vary widely among samples. In juvenile trees, the amounts of other internal regulators may be quite different from those in mature trees. More studies of endogenous phytohormones in juvenile trees should be done for the better understanding of xylem formation in juvenile trees.

In sugi trees, it was previously reported that MFA in earlywood was relatively constant in the radial direction while MFA in latewood dramatically decreases in the radial direction (from pith to bark) [25]. Using the MFA obtained in our previous study [26], the ratio of MFA in mature to juvenile wood was calculated both in earlywood and latewood. It was recognized that the radial variations of MFA in earlywood were less than those in latewood in Obi-sugi cultivars. In some sugi cultivars, MFA in earlywood did not change between juvenile and mature wood. The IAA amounts in cambial-region tissues in spring (during earlywood formation) are assumed to be larger than those in September, based on the seasonal variation of IAA amounts of pine trees [12, 13]. The potentially larger IAA amounts during earlywood formation may affect the variation of MFA in earlywood. To elucidate the effects of IAA amounts on MFA, in a growing season, seasonal variation of MFA and IAA amounts should be examined in juvenile and mature trees in the future study. In juvenile pine trees, it was reported that a higher *H*/*D* ratio and greater tree height increased the modulus of elasticity (MOE) of 4-year-old radiata pines [5, 6] and 8-year-old slash pines [7], respectively. It is well known that smaller MFAs improved the mechanical properties of wood. Therefore, from these studies on juvenile pine trees, it was assumed that trees with larger tree height and larger H/D ratio had smaller MFAs in the stems. In this study, it was recognized that IAA amounts in the lower trunk decreased with tree height (Fig. 2), and samples with small amounts of IAA had smaller MFA than the samples with very large amounts of IAA (Table 2). These results are consistent with the results of studies on juvenile pine trees [5–7]. For the efficient selection of vigorous hybrid poplar, the relationship between gibberellin (GA) concentration and shoot growth in juvenile hybrid poplar was examined [27, 28]. In juvenile sugi trees, decrease of IAA amounts in lower trunk by selection of genotype and silvicultural treatment (pruning and large planting densities) may improve the juvenile wood properties for production of the superior wood as structural materials.

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